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Biologically Enhanced Dissolution of Tetrachloroethene DNAPL

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One major problem with tetrachloroethene (PCE) contamination of aquifers is its ability to form dense, nonaqueous-phase liquids (DNAPL), which can act as a persistent contamination source for decades. Batch studies were performed to determine the potential for biological reductive PCE dehalogenation at high concentration and the effect on competing microorganisms, including methanogens and homoacetogens. Results show that PCE dehalogenation can be obtained at saturation concentration (>0.9 mM). Also, trichloroethene was dehalogenated up to 2.26 mM, and no apparent inhibitory effect on dehalogenation was found with *cis*-1,2-dichloroethene (cDCE) and ethene at the highest tested levels of 0.66 and 1.05 mM, respectively. However, such high concentrations of PCE, cDCE, and ethene were inhibitory to methanogens, and high concentrations of PCE were inhibitory to homoacetogens. Such inhibition is highly beneficial as it greatly diminished the competition by methanogens and homoacetogens for added electron donors, including hydrogen, resulting in highly efficient substrate utilization for dehalogenation. PCE DNAPL dehalogenation in a column study required less than 1 g of the electron donor pentanol to dehalogenate 1 g of PCE to cDCE (<2 mol of pentanol/mol of PCE). Additionally, DNAPL dissolution rate was significantly enhanced when directly coupled with biological dehalogenation.

Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) are common groundwater contaminants. One major problem associated with these solvents is their ability to form dense, nonaqueous-phase liquids (DNAPLs) (*1*−*3*). When sufficient quantities are present, the resulting PCE or TCE DNAPLs can penetrate through permeable groundwater aquifers and pool at a nonpermeable zone where they can provide a long-term source of groundwater contamination. The time required for complete dissolution for a typical accumulation of the solvents can be hundreds of years under natural conditions (*4*).

Because most of the contaminant mass at a DNAPL site generally resides in the source zone, there have been numerous attempts to control, confine, or remove the source zone DNAPL. The main technologies available at present include pump-and-treat, low-permeability enclosures, vapor stripping, solvent or detergent extraction, and chemical oxidation (*5*, *6*). Although there have been many studies on anaerobic PCE and TCE biodegradation (*7*−*11*), it has generally been regarded that microbiological methods are only effective in treating the resulting plume from DNAPL dissolution rather than the DNAPL itself (*5*, *6*). The main reason for this belief is that saturation concentrations are likely to be toxic to dehalogenating bacteria (*12*). For instance, PCE dehalogenation by *Dehalospirillum multivorans* was inhibited when PCE concentration was higher than 0.3 mM (*13*), well below the saturation concentration of about 0.9 mM (*3*, *6*). In another study, a mixed culture acclimated to high PCE concentrations was inhibited by PCE at 0.55 mM (*14*). However, a few recent studies suggest that reductive dehalogenation of DNAPLs or at least at the high concentrations existing near a DNAPL source can occur. Facultative strain MS1 was found capable of transforming a near-saturation concentration of PCE (*15*). Nielsen and Keasling (*16*) reported that a microbial culture enriched from a TCE-contaminated groundwater aquifer could perform dehalogenation of saturated PCE at a faster rate than under subsaturating conditions. Such reports justify further exploration of DNAPL biological dehalogenation.

Another factor that adversely affects the practical application of biological dehalogenation of PCE and TCE is competition for added electron donors by other microorganisms (*14*, *17*). Electron donor substrates are generally fermented anaerobically to produce hydrogen that is used by methanogens, homoacetogens, and sulfidogens as well as the dehalogenating microorganisms of interest. Thus, more economical application of anaerobic reductive dehalogenation might be realized by conditions that favor the dehalogenators over their competitors (*18*). High concentrations of PCE are toxic to methanogens and acetogens (*19*). This suggests that the issue of competition might be reduced with DNAPL biodegradation. In addition, Seagren et al. (*20*, *21*) indicated that biodegradation of NAPL should increase NAPL dissolution rate, leading to faster cleanup.

In this paper, we report factors affecting the dehalogenation of high concentrations of PCE and TCE using an anaerobic mixed culture. The culture contained four groups of microorganisms: fermenters, homoacetogens, methanogens, and dehalogenators, the last three of which compete for hydrogen.

Materials and Methods

Chemicals. Liquid PCE (99.9+%, HPLC grade, Aldrich Chemical Co., Milwaukee, WI), TCE (99+%, spectrophotometric grade, Aldrich Chemical Co.), and cDCE (97%, Aldrich Chemical Co.) were used for preparing stock feed solutions and analytical standards. Vinyl chloride (VC), ethene, and methane gases (99+%, Scott Specialty Gases, Alltech Associates, Inc., Deerfield, IL) were used as analytical standards. Pentanol (99%, Aldrich Chemical Co.), benzoate (sodium salt, 99%, Aldrich Chemical Co.), and hydrogen (99.99%, Scott Specialty Gases) were used as electron donors and to develop analytical standards. Acetate (analytical reagent, J. T. Baker Chemical Co., Phillipsburg, NJ) was used for analytical standards.

Culture and Growth Medium. The dehalogenating culture used in the study has been maintained in a closed continuously stirred tank reactor (CSTR) (total volume 4.3 L, liquid volume 3.6 L) initially seeded with aquifer material from a PCE-contaminated groundwater site in Victoria, TX. The reactor was maintained at 28 (± 2) °C. A continuous anaerobic feed consisting of 1.7 mM sodium benzoate, 20 mg/L yeast extract, 0.98 mM PCE, and trace nutrients in basal medium (*17*) was syringe-pumped at 100 mL/day, resulting in a 36-day detention time. Essentially complete conversion of PCE to ethene was obtained. The steady-state microorganism

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TABLE 1. Solubility and Henry's Law Constants at 25 °C

	solubility (mM)	Henry's law constant (dimensionless)
PCE	0.9[a]	0.716[b]
TCE	8.4[a]	0.386[b]
cDCE	36[a]	0.154[b]
VC	43[a]	1.077[b]
ethene	4.7[a]	8.5[a]

[a] Yaws (*32*). [b] Gossett (*33*).

concentration was 10 mg/L measured as volatile suspended solids (VSS) (*17*).

Batch Experiments. All batch experiments except one using biogenic cDCE were conducted at room temperature (22 °C) using batch conditions with 120-mL serum bottles, to which 60 mL of the dehalogenating culture was added anaerobically while gas purging with a 80% N_2/20% CO_2 mixture. In the first study on dehalogenation of various concentrations of PCE, different amounts of pure PCE were added to create a set of initial aqueous concentrations of 0.26, 1.06, 2.11, and 4.22 mM (corrected for vapor-phase concentration). However, since PCE solubility is about 0.9 mM at the temperature used (see Table 1), bottles with higher concentrations contained the excess PCE as a separate DNAPL phase. Pentanol (7.67 mM) was used as the substrate in these two experiments. Below 10 mM, it has no apparent inhibitory effect on the dehalogenating microorganisms. Pentanol was measured regularly to ensure the presence of excess electron donor. A similar study was conducted with TCE of 0.14, 0.28, 0.56, 1.13, and 2.26 mM (aqueous concentration). The highest TCE concentration used is below saturation level (Table 1). Pentanol (3.07 mM) was used as the substrate.

The effect of PCE on the bacterial community was investigated with 0.1, 0.3, and 1.0 mM PCE aqueous concentration. Here, hydrogen (0.37 mmol) was added as the substrate. Considering the excessive toxicity of commercial cDCE reported by S. Zinder (personal communication) and confirmed by our previous studies, biogenic cDCE was used to investigate the effect of cDCE concentration on bacterial community response. Biogenic cDCE was generated by adding PCE periodically to a culture of strain MS1, which is known to dehalogenate PCE to cDCE (*15*). MS1 was grown on acetate and yeast extract in a 160-mL bottle containing 100 mL of a basal medium (*17*) until about 2.2 mM cDCE had been produced. Different amounts of the solution were transferred by syringe into 60-mL serum bottles to create a set of initial cDCE concentrations of 0.11, 0.18, 0.34, and 0.66 mM. Various amounts of biogenic cDCE solution sparged free of cDCE were also added at the same time so that the total amount of biogenic solution present was the same in all bottles. The final liquid volume was 35 mL, which included 14 mL of inoculation from the CSTR culture. Benzoate (0.067 mmol) was used as the substrate in this study.

The effect of ethene on bacterial community was examined at different levels: 0, 0.005, 0.016, 0.053, 0.16, and 1.05 mM (aqueous concentration). Three parallel batch studies were conducted: with PCE, with cDCE, and with no chlorinated ethene. Hydrogen (0.19 mmol) was here used as the substrate. Duplicates were prepared for each of the above experiments and had a variability within 10%.

To maintain anaerobic conditions, rubber stoppers (Bellco Glass Inc., Vineland, NJ) and aluminum crimp caps were used to seal the bottles. Adsorption of chlorinated compounds on the rubber stoppers was less than 5% after the first few days so that good mass balances could be maintained throughout the studies. All batch bottles were continuously mixed at 100 rpm on a shaker table (Lab-Line Instruments, Inc., Melrose Park, IL).

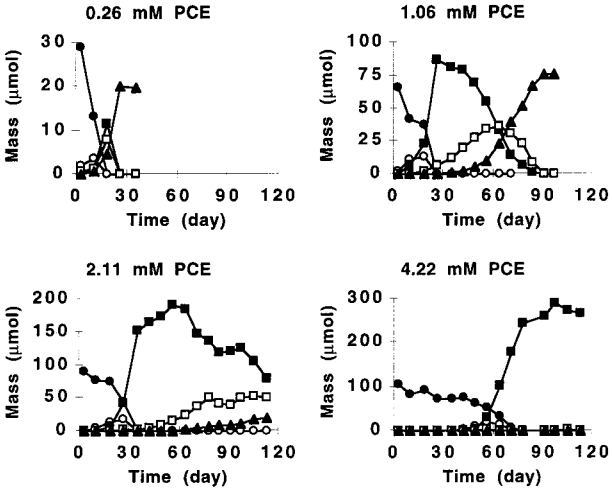

FIGURE 1. Dehalogenation of sub- and supersaturated PCE (●). Transformation products were TCE (○), cDCE (■), VC (□), and ethene (▲). Note the mass values here were calculated according to the headspace concentration measured, and the amount of PCE, TCE, cDCE, VC, and ethene before disappearance of PCE DNAPL did not represent the actual amount of these compounds because of the possible partitioning.

Column Study. An upflow glass column (25 mm i.d. by 220 mm) operated at room temperature had the lower 70% filled with aquifer material and the upper 30% filled with the CSTR culture. The aquifer material, originally from an uncontaminated groundwater site at Moffett Federal Airfield, Mountain View, CA, was amended with PCE by syringe to about 2% saturation of the pore space while adding aquifer material as uniformly as possible. The column was then continuously fed with a sterile basal medium (*17*) containing 1.8 mM pentanol during the first 70 days of operation (3.7 mM afterward) by a syringe pump at a rate of 4.5 mL/day, resulting in a liquid detention time of 14 days. The outgoing tubing, fittings, sampling syringes, and vials were made of Teflon or stainless steel to minimize sorptive loss. Effluent from the column was collected in a 5-mL glass syringe barrel with a tight-fitting Teflon float to prevent volatilization losses, 1.0 mL of which was then transferred to a 5-mL glass vial. After vigorously shaking the vial for about 10 min, the headspace was extracted to perform degradation product measurements.

Analytical Methods. PCE, TCE, cDCE, VC, ethene, and methane were quantified with gas chromatography (GC) as described elsewhere (*17*). Acetate and benzoate analyses were performed with ion chromatography (IC). 1-Pentanol was measured with a 5890 series II GC (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) and a Nukol column (30 m × 0.25 mm, Supelco, Inc., Bellefonte, PA). Identification and quantification were made by comparison with external standards.

Results

PCE Dehalogenation. PCE dehalogenation occurred at all concentrations tested from 0.26 to 4.22 mM (Figure 1). The mass values shown represent computed liquid plus headspace masses only as derived from headspace measurements and Henry's law constants (Table 1). The PCE mass represented by DNAPL and the mass of other ethenes dissolved in the PCE DNAPL are thus not included. With the two higher PCE initial concentrations, a significantly higher mass of cDCE accumulated than the starting PCE liquid plus headspace mass, which clearly shows the effective removal of PCE

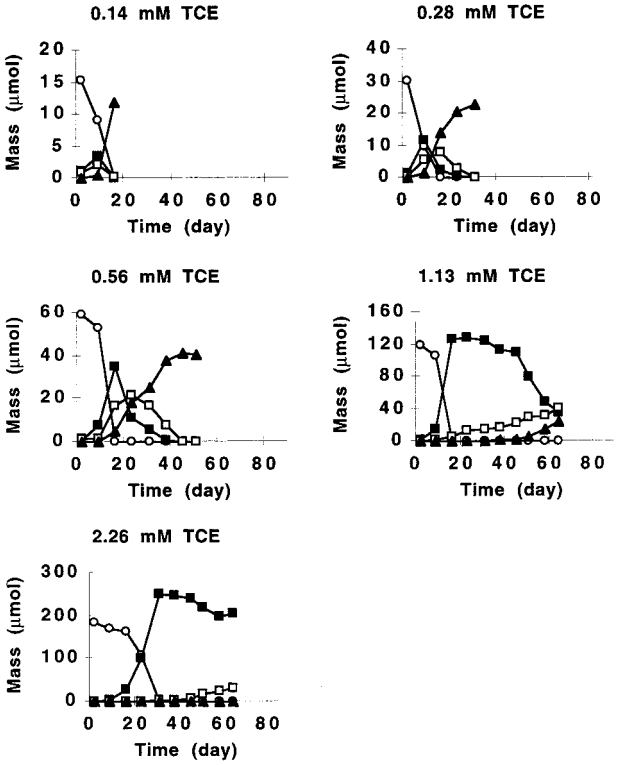

FIGURE 2. Dehalogenation of TCE (○). Transformation products were cDCE (■), VC (□), and ethene (▲).

DNAPL through dehalogenation. Within 4 months, complete dehalogenation to ethene was obtained with the two lower PCE concentrations of 0.26 and 1.06 mM. Almost no lag time was observed except at the highest PCE concentration of 4.22 mM, where some toxicity is suggested. Since the initial near-solubility PCE aqueous concentration was about the same with 4.22 mM as with 1.06 or 2.11 mM, the inhibition with 4.22 mM might be related to a greater time of exposure to high concentration or to some effect from direct contact with DNAPL.

TCE Dehalogenation. All TCE concentrations shown in Figure 2 were below TCE solubility concentrations (Table 1), and so no TCE DNAPL was present here. TCE was readily dehalogenated at concentrations from 0.14 to 2.26 mM. With the three highest concentrations shown in Figure 2, a significant increase of TCE dehalogenation rate was observed after about 1−2 weeks. The lag period tended to increase with an increase in initial TCE concentration, as confirmed in other experiments with even higher TCE concentrations (data not shown). However, in other experiments only a trace amount of cDCE was observed when TCE DNAPL was used, yielding a solution TCE concentration of 8.4 mM (data not shown). Further study is required to determine whether acclimation to DNAPL TCE concentrations can be achieved by dehalogenators.

Effect of PCE on the Bacterial Community. The effect of PCE on methanogenesis and homoacetogenesis is shown in Figures 3 and 4. Here, hydrogen was added as the substrate. In the absence of PCE, methane production was complete within 5 days, and only a small amount of acetate was formed (Figure 3), suggesting that the methanogens in the culture were very active. In the presence of PCE, dehalogenation was incomplete with cDCE and/or VC remaining although the hydrogen added was sufficient for complete transformation of 1 mM PCE to ethene. With 0.1 mM PCE, only about 6 μmol of methane was produced during the first 5 days when PCE was present. Following PCE disappearance, methane quickly rose to 76 μmol. No acetate was formed,

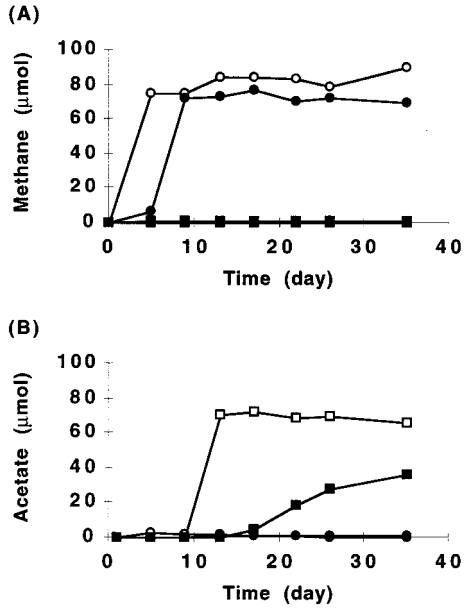

FIGURE 3. Concurrent methane (A) and acetate (B) production during PCE dehalogenation with hydrogen, no PCE control (○), 0.1 (●), 0.3 (□), and 1.0 mM (■).

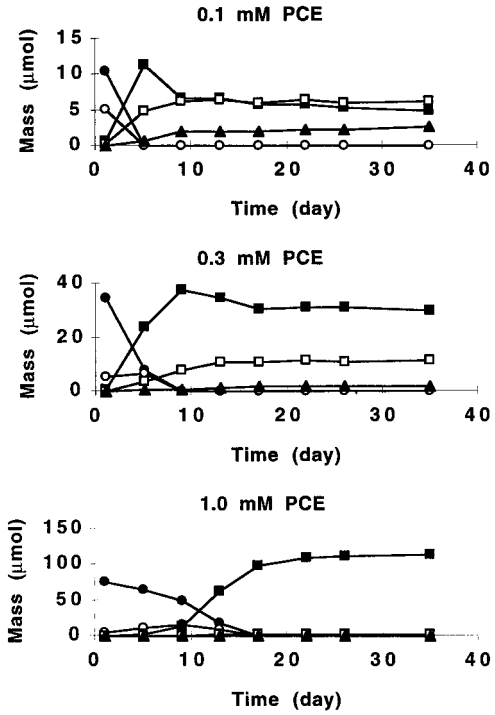

FIGURE 4. Dehalogenation of PCE (●) with hydrogen to TCE (○), cDCE (■), VC (□), and ethene (▲).

indicating that homoacetogens had insufficient time to form a significant level of acetate from hydrogen and carbon dioxide. With a PCE concentration of 0.3 mM, however, only a small amount of methane was produced, whereas acetate was quickly produced (Figure 3) as soon as PCE disappeared (Figure 4) and rose to about 1.2 mM after 15 days without significant change thereafter. A further increase of PCE to 1.0 mM produced similar results as with 0.3 mM PCE, except that acetate was formed at a much slower rate.

The fact that no significant methane or acetate production was observed before the disappearance of PCE suggests that concentrations of PCE even as low as 0.1 mM can inhibit methanogenesis and homoacetogenesis. Homoacetogens

TABLE 2. Mass Balance of PCE Dehalogenation with Hydrogen

PCE concn (mM)	total dehalogenation[a] (as μmol of H_2 equiv)	methane (μmol)	acetate (μmol)	H_2 consumed[b] (μmol)	utilization efficiency for dehalogenation	H_2 input[c] (μmol)
0.1	39	77	1	351	11%	370
0.3	100	1	66	368	27%	370
1.0	235	0	36	379	62%	370

[a] Calculated based upon the amount of dehalogenation products TCE, cDCE, VC, and ethene, which represent 1, 2, 3 and 4 μmol of H_2 equiv/μmol, respectively. [b] Based on 4 μmol of H_2 equiv/μmol of methane and acetate formed. [c] 20 μmol of H_2 equiv derived from control bottles was included as part of input.

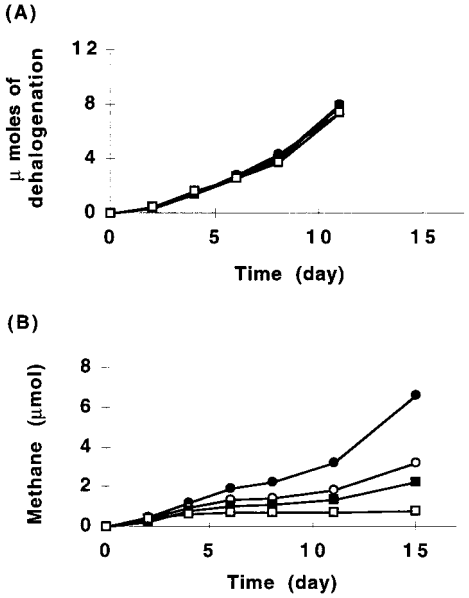

FIGURE 5. Dehalogenation of various concentrations of biogenic cDCE: 0.11 (●), 0.18 (○), 0.34 (■), and 0.66 mM (□). (A) Total dehalogenation in μmol of chloride released as calculated from μmol of VC and ethene formed. (B) Methane production.

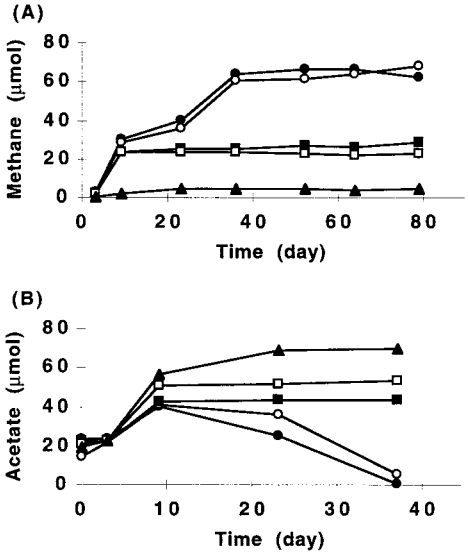

FIGURE 6. Effect of various concentrations of ethene: 0 (●), 0.005 (○), 0.016 (■), 0.053 (□), and 0.16 mM (▲). (A) Methane production and (B) acetate concentration.

appear to have been inhibited somewhat less than methanogens by PCE for when 0.3 mM PCE was added, acetate was produced following PCE disappearance, but methane was not. The noted inhibitory effect of PCE on methanogens is consistent with previous observations (*16*, *19*). The differential production of methane and acetate after PCE was transformed could result either from a long-term toxic effect of PCE on methanogens and homoacetogens or from an inhibitory effect of the high concentrations of cDCE produced.

Since high concentrations of PCE diminished the activity of methanogens and homoacetogens, it was expected that the substrate utilization efficiency for dehalogenation would rise. A mass balance showed this to be the case. Table 2 indicates that the efficiency of hydrogen used for dehalogenation was 11, 27, and 62% for 0.1, 0.3, and 1.0 mM PCE, respectively. However, active methanogenesis or homoacetogenesis occurred after PCE disappeared, and thus insufficient hydrogen was available for complete dehalogenation to ethene.

Effect of cDCE on the Bacterial Community. Since cDCE is used slower than PCE and TCE and often accumulates during PCE dehalogenation, the effect of biogenic cDCE on dehalogenation was evaluated over a range from 0.11 to 0.66 mM. No significant difference in the mass rate of dehalogenation was observed (Figure 5A), indicating that cDCE was not inhibitory to dehalogenation over this concentration range. In contrast, methane production decreased markedly with an increase in cDCE concentration (Figure 5B). Only about 10% as much methane was produced with 0.66 mM cDCE as with 0.11 mM cDCE. Since substrate utilization for dehalogenation was the same over the first 8 days regardless of initial cDCE concentration, reduced methane production with increase in cDCE concentration might result from either direct inhibition of methanogens or inhibition of benzoate fermentation, making less substrate available for methanogens. Since benzoate was used similarly in all bottles (data not shown), inhibition of methanogenesis is suggested.

Effect of Ethene on the Bacterial Community. Ethene is the usual final product of dehalogenation and has been shown to inhibit methanogenesis (*22*−*24*). As illustrated in Figure 6A, methane production occurred in two stages at low ethene concentration, hydrogen-utilizing (autotrophic) methanogenesis occurred during the first week and acetate-utilizing (acetoclastic) methanogenesis after that. The latter is supported by the associated decrease in acetate concentration during this time as indicated in Figure 6B. Acetate formation from hydrogen through homoacetogenesis between day 4 and day 10 is also indicated in Figure 6B by the increase in acetate concentration during this period. The near simultaneous utilization of hydrogen for both methane and acetate formation during the first 10 days here is different from the previous experiment where the sole use of hydrogen was for methanogenesis as shown for the control in Figure 3. The reason for the difference is not clear, but the cultures for the two experiments were taken from the reactor at quite different times, and the relative populations of the two groups of hydrogen utilizers may have been different.

Figure 6B indicates that increasing levels of ethene did not appear to inhibit homoacetogens as acetate was produced by day 10 in all bottles. However, with increase in ethene concentration to 0.016 and 0.053 mM, only autotrophic methanogenesis was observed, but at a slightly reduced level than in the control. An absence of acetoclastic methanogenesis at these ethene concentrations is indicated by the

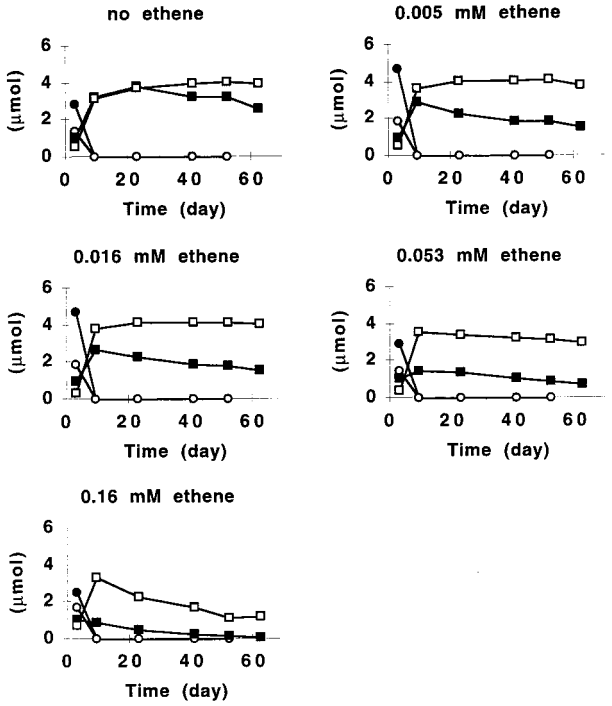

FIGURE 7. Effect of ethene on PCE (●) dehalogenation. Transformation products were TCE (○), cDCE (■), and VC (□).

lack of decrease in acetate concentration between day 10 and day 37. Thus, acetoclastic methanogens appear to be more sensitive to ethene than autotrophic methanogens, as suggested previously (*17*). A further increase of ethene to 0.16 mM resulted in almost complete cessation of both classes of methanogenesis. Also as shown in Figure 6B, an increasing amount of acetate was produced with an increasing level of ethene, which is also attributed to the ethene inhibition of both autotrophic and acetoclastic methanogenesis.

The effect of ethene on dehalogenation of PCE and cDCE was also evaluated. No inhibitory effect on dehalogenation of PCE was observed with any concentration of ethene (Figure 7). Although PCE dehalogenation to ethene was incomplete in all cases, the lower concentration of cDCE and VC with higher ethene concentration confirms that more dehalogenation occurred at the higher ethene concentrations. This suggests that higher ethene concentrations aid dehalogenation by making more substrate available for dehalogenation by inhibiting methanogenesis. A similar effect of ethene on cDCE dehalogenation was also observed (data not shown).

A separate study on PCE dehalogenation with 1.05 mM ethene (partial pressure 0.2 atm) also showed no inhibitory effect on PCE dehalogenation (data not shown). This ethene concentration corresponds to that found from complete dehalogenation of a saturated PCE solution.

Column Study. PCE DNAPL dehalogenation was further examined with a continuous-flow column. The concentration change of PCE and end products formed in the effluent over time for the column study is shown in Figure 8. Efficient dehalogenation of PCE solubilized from PCE DNAPL is indicated by the accumulation of intermediates, mainly cDCE. After a time period equivalent to three detention times, the concentration of PCE plus end products totaled more than the saturation concentration of PCE (0.9 mM). After about 60 days of operation, cDCE production started to level off, appearing to arrive at a steady-state, but no significant amount of VC and ethene was produced. The total concentration of the chlorinated ethenes was about 2.3 mM. From day 70, the pentanol concentration in the feed was doubled

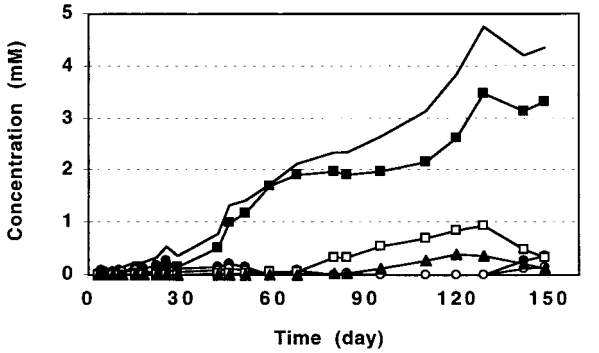

FIGURE 8. Concentration of PCE (●), TCE (○), cDCE (■), VC (□), ethene (▲), and the total (without marker) in the column effluent with time.

from 1.8 to 3.7 mM. Soon cDCE began to be dehalogenated, accompanied by almost complete disappearance of PCE and TCE in the effluent and formation of VC and ethene. It was also accompanied with further acceleration of cDCE production until day 130 when cDCE started to level off. The total concentration of the chlorinated ethenes and ethene was then about 4.5 mM. This represents about 5 times the PCE solubility concentration. This suggests dehalogenation resulted in a significantly enhanced PCE dissolution rate as predicted by the models of Seagren et al. (*20, 21*).

One puzzle associated with the column results is the lack of mass balance during the quasi-steady-states. Only about 50% of hydrogen expected from substrate pentanol fermentation (7 mol of H_2/mol of pentanol) was represented by dehalogenation (46% by dehalogenation and 4% by methanogenesis). One possibility for the lack of mass balance is that the system had not yet reached steady-state. A significant amount of the cDCE produced can partition back into the PCE DNAPL phase (*16*). This would lead to underestimating the actual amount of cDCE produced.

Discussion

A major interest of this study was to evaluate the possibility of applying anaerobic reductive dehalogenation to PCE at high concentrations. Several observations made here suggest that there are significant advantages and also challenges to applying reductive biological dehalogenation directly to the DNAPL source zone.

First, the Victoria culture used here was able to transform PCE at saturation concentration. Although direct contact with PCE DNAPL showed some inhibitory effect, high concentrations near DNAPL could readily be accomplished. Dehalogenation of saturated solution has been reported by others (*15, 16*). Nielsen and Keasling (*16*) noted an increase in PCE dehalogenation rate with increase in concentration to near 1 μmol (mg of protein)$^{-1}$ h^{-1} at PCE saturation. A similar increase in rate with increase in concentration to a maximum rate of 0.42 μmol (mg of protein)$^{-1}$ h^{-1} (assuming VSS is 50% protein) was found here.

Second, TCE and ethene were shown to have no apparent inhibitory effects on PCE and TCE dehalogenation at concentrations observed during saturated PCE dehalogenation. However, as found in the column study, a high concentration of cDCE (3.5 mM) resulted from PCE DNAPL dehalogenation. Such accumulation might result from one or a combination of four factors: PCE toxicity to cDCE and VC dehalogenation, cDCE toxicity to cDCE and VC dehalogenation, slow cDCE dehalogenation kinetics (25), or competition between different dehalogenation steps for available electron donor. Because cDCE is a regulated contaminant, more studies are necessary to pinpoint the reasons for such accumulation and to investigate the

measures that can be taken to achieve overall successful bioremediation of PCE DNAPL. Even if dehalogenation of PCE is incomplete, there are possible advantages to its conversion to cDCE and VC. The enhanced dissolution of PCE and high concentrations of cDCE that results can reduce overall time for cleanup as well as increase the effectiveness of downgradient treatment systems, such as pump-and-treat. Additionally, the dehalogenation products from PCE are biodegradable through aerobic processes, such as cometabolism. Thus, other in situ biodegradation processes can be used to complete the removal process.

Third, the problem of competitive utilization of added electron donor substrate is greatly alleviated with saturated solution PCE dehalogenation. This is due to the inhibitory effect of high concentrations of PCE, cDCE and also the end product ethene on competing methanogens and homoacetogens. This is highly beneficial to in situ bioremediation for two reasons. One is that the resulting electron donor utilization efficiency for dehalogenation is very high. This overcomes one of the greatest cost factors for reductive dehalogenation. The second is that a large range of electron donors can be efficiently used when competition is eliminated. Slow hydrogen release substrates are not necessary for high utilization efficiency. Indeed, hydrogen gas itself was here used very efficiently for reductive dehalogenation, supporting the potential usefulness of direct hydrogen addition as have been proposed (*26*). In the column study reported here, less than 1 g of pentanol was required to dehalogenate 1 g of PCE to cDCE.

Additionally, a markedly higher concentration of PCE plus its dehalogenation products as compared with PCE solubility concentration was observed in the effluent of the continuously fed column, which indicates up to a 5-fold increase in PCE DNAPL dissolution rate than could occur in the absence of biological dehalogenation. The dissolution process is one of the key factors controlling subsurface remediation of NAPLs. Many approaches have been studied to enhance dissolution, such as flushing (*27*) and surfactant washing (*28*). But almost no experimental evidence for biological enhancement of NAPL dissolution by solute degradation exists. This mechanism was proposed as an explanation for experimental observations of the microbial enhancement of desorption of biodegradable organic compounds from solids (*29*, *30*) and dissolution of solid organic compounds (*31*). Seagren etc. (*20*, *21*) quantitatively modeled the residual NAPL and NAPL-pool dissolution rate by flushing and biodegradation and suggested that biodegradation can act as a reaction sink to increase the concentration gradient and, thus, increase the NAPL dissolution rate. The results here provide experimental validation for this, confirming the significant potential of this technique to enhance PCE dissolution from DNAPL and thus increase the speed of cleanup.

Acknowledgments

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